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Yeast species from soil and fallen leaves new for the mycobiota of Israel

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Abstract — We investigated the species diversity of yeasts from soil and fallen leaves of Israel based on sequencing of the D1/D2 domain of 26S rDNA. Seven new yeast records found for Israel were *Apiotrichum nothofagi, Cryptococcus carnescens*, *C. phenolicus*, *C. terreus*, *Komagataella pastoris, Rhodosporidium lusitaniae*, and *Schwanniomyces occidentalis*.

Key words — *Ascomycota*, *Basidiomycota*, Middle East, molecular methods

Introduction

The biodiversity of yeasts in Israel has not been studied well. There has not been any critical research regarding the biodiversity of this group of fungi. One thorough investigation was done at the "Evolution Canyon" microsite in Mount Carmel by Nagornaya et al. (2003). Some selective studies were dedicated to the biodiversity of yeasts in hypersaline water habitats (Butinar et al. 2005). *Yarrowia lipolytica* was found together with *Rhodotorula glutinis* on salt-excreting leaves of *Atriplex halimus* plants in the central Negev Highlands of Israel (Zvyagilskaya et al. 2001). Many yeast species were collected in vineyards in the Coastal Plain of Israel (Zahavi et al. 2002), and *Metschnikowia fructicola* was first described from grape surfaces in Israel (Kurtzman & Droby 2001). *Metschnikowia reukaufii* was isolated from pollen of *Asclepias syriaca* (Eisikowitch et al. 1990). *Clavispora lusitaniae* was first described from citrus essence in Israel (Rodrigues de Miranda 1979). Several yeast species have been recorded on mites, in citrus products and organic acid media, on *Zea mays*, and on grapefruit (Smith 1986, Steiman et al. 1997, Schena et al. 2000, Boekhout et al. 2003, Savchenko et al. 2010, Kurtzman et al. 2011).

Forty-two yeast species are reported in the literature from Israel: 24 ascomycetes (in 15 genera and eight families) and 18 basidiomycetes (in nine genera and eight orders).

We investigated yeast species diversity of soil and fallen leaves based on sequencing of the D1/D2 domain of 26S ribosomal DNA in order to achieve better insight into the biodiversity of yeast species in Israel.

Materials & methods

Collection site

Soil and fallen leaves samples were collected in En Ziwan, 33°11′09″N 35°79′10″E, Golan Heights; Carmel National Park, 32°75′00″N 35°02′70″E, Carmel Mount; and Kefar Kisch, 32°65′32″N 35°45′06″E, Lower Galilee.

Isolation techniques

To isolate the yeasts from soil and fallen leaves, 10 g of each sample was diluted in 100 ml of sterile double-distilled water and placed on the orbital shaker for one hour at 150 rpm at room temperature. After shaking, each sample was diluted decimally; 0.15 ml of each dilution was spread on acidified yeast-malt (YM) agar (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% agar) pH 3.7–3.8, or YM agar containing chloramphenicol (0.01%) and sodium propionate (0.2%) for suppression of bacterial and mold growth. The plates were incubated at 25°C for 3–7 days. Representative colonies of each morphology type were picked and streaked onto new YM agar plates. The purity of yeast cultures was ascertained using light microscopy. Color names follow Rayner (1969). The yeast cultures were stored at 4°C in YM agar plates. They are available from culture collection of the Institute of Evolution, University of Haifa (HAI, Haifa, Israel). For long-term preservation they were frozen at –80°C in 25% glycerol YM medium.

Molecular-biological methods

Yeasts were identified taxonomically using molecular-biological methods. DNA was extracted according to Hoffman & Winston (1987) with Gottschling (http://labs.fhcrc.org/gottschling/Yeast%20Protocols/qgprep.html) and Dunham (http://dunham.gs.washington.edu/MDyeastDNAprep.htm) modifications. DNA concentration was measured using the NanoDrop®ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, U.S.A.). The concentration of DNA was adjusted to 10 ng/µl for further applications. Diluted DNA (10ng/µl) was used as a PCR template. The D1/D2 domain of the large subunit (26S) of ribosomal DNA (rDNA) was amplified on GeneAmp® PCR System 9700 (Applied Biosystems, USA) using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell 1993), and ABgene® Thermo Scientific 2X ReddyMix PCR Master Mix w/1.5 mM $MgCl_2$ (Applied Biosystems, California, USA). PCR cycling conditions included initial denaturation (95°C for 5 min), 5 extension cycles (94°C for 45 s, primer annealing at 55°C for 45 s, primer extension at 72°C for 1 min), 29 denaturation cycles (95°C for 30 s, primer annealing at 60°C for 30 s, primer extension at 72°C for 1 min), and a final elongation step at 72°C for 10 min. PCR products were visualized in 1.5% agarose gel, stained with GelRedTM Nucleic Acid Gel Stain, 10,000× in water (Biotium, Inc.,

Hayward, California), and viewed under UV. PCR products were cleaned using an ExoSAP-IT (USB Corp.) following the manufacturer's instructions.

The PCR products were sent to the Technion (Israel Institute of Technology, The Bruce Rappaport Faculty of Medicine) Medicine Lab for sequencing, which was performed on 3130xl Genetic Analyzer (Applied Biosystems, USA) using the PCR primers and Big Dye® Terminator v 1.1 Cycle (Applied Biosystems, USA). We identified the yeast species in a BLAST search. BLASTN 2.2.26+ program was to align sequences and compare them with type strains, percentage of query coverage, and max identity (Zhang et al. 2000). All sequences were registered in GenBank.

Results

Analysis of the 26S rDNA D1/D2 sequences revealed seven yeast species not previously reported for Israel, of which two are ascomycetes (*K. pastoris, S. occidentalis*) and five are basidiomycetes (*A. nothofagi*, *C. carnescens*, *C. phenolicus*, *C. terreus*, *R. lusitaniae*).

Apiotrichum nothofagi C. Ramírez & A.E. González, Mycopathologia 88(2–3): 76 (1984) [as "*Apiotricum*"]. Fig. 1

Type: Chile, Futrono, in evergreen rainy Valdivian forest, from decayed wood of *Nothofagus obliqua*, 1980, A. González (IJFM 6018).

≡ *Rhodotorula nothofagi* C. Ramírez & A.E. González, Mycopathologia 91(3): 171 (1985), nom. illegit. [superfluous sp. nov., based on same type].

≡ *Rhodotorula nothofagi* (C. Ramírez & A.E. González) Roeijmans, Eijk & Yarrow, Mycotaxon 35(2): 406 (1989), nom. illegit. [superfluous, later homonym].

Cells after 3 days growth on YM agar at 25°C subglobose to ellipsoid, 3–4 × 6–11 µm, occurring mostly singly or in pairs. Budding predominantly polar. Colonies white and smooth.

Specimens examined – **ISRAEL. Carmel Mount**, Haifa, Carmel National Park, 32°75′18″N 35°02′69″E, 18.XII.2010, from fallen needles of *Pinus halepensis*, leg. D.M. Gotman (HAI-Y-35; GenBank JQ581048); **Lower Galilee**, Kefar Kish, 32°65′32″N 35°45′06″E, 22.XII.2010, from fallen leaves of *Ceratonia siliqua*, leg. D.M. Gotman (HAI-Y-81; GenBank JQ581049).

Comments – *Apiotrichum nothofagi* belongs to the *Curvibasidium* clade of *Microbotryomycetes*. A single strain IJFM 6018 (CBS 8166) was isolated in 1980 from decayed wood of *Nothofagus obliqua*. In 1984, Ramírez & González described a new taxon, *Apiotrichum nothofagi*, citing IJFM 6018 as type culture. The following year, they published *Rhodotorula nothofagi* as a new species, also citing IJFM 6018 as type culture, rendering *R. nothofagi* a superfluous and illegitimate name (ICBM [Vienna Code] Art. 52.1). Apparently unaware that *R. nothofagii* had already been validly published by Ramírez & González, Roeijmans et al. (1989) recombined *A. nothofagi* as *Rhodotorula nothofagi*, a comb. nov. that is also a superfluous and illegitimate later homonym. We cite this taxon here under its only legitimate name, *Apiotrichum nothofagi*, but it

requires a nom. nov. in *Rhodotorula*, since both existing *Rhodotorula* names are illegitimate.

Distribution – Europe, North America, South America (Gadanho et al. 2003, Maksimova & Chernov 2004, Villa-Carvajal et al. 2004, Langdon et al. 2005, Rodrigues et al. 2009, Golubev & Tomashevskaya 2010).

Cryptococcus carnescens (Verona & Luchetti) M. Takash. et al., Int. J. Syst. Evol. Microbiol. 53(4): 1192 (2003) Fig. 2

Cells after 3 days growth on YM agar at 25°C single, ellipsoidal or subglobose, 3–7 µm; budding monopolar. Colonies [D]-colored, smooth.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′09″N 35″79′10″E, 11.I.2011, from fallen leaves of *Quercus boissieri*, leg. D.M. Gotman (HAI-Y-183; GenBank JQ317682).

Comments – *Cryptococcus carnescens* belongs to the *Tremellales* (*Tremellomycetes*).

Distribution – Europe, Asia, Antarctica (Gadanho et al. 2003, Wuczkowski et al. 2005, Butinar et al. 2007, Connell et al. 2008, Golubev & Tomashevskaya 2010, Li et al. 2010, Loque et al. 2010, Kurtzman et al. 2011, Baleiras-Couto et al. 2012).

Cryptococcus phenolicus Á. Fonseca et al., Can. J. Microbiol. 46(1): 24 (2000) Fig. 3

Cells after 3 days growth on YM agar at 25°C single, globose, 4–8 µm in diam; budding monopolar. Colonies [B]-colored with glossy and smooth surfaces.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′16″N 35″79′10″E, 11.I.2011, from grassland soil, leg. D.M. Gotman (HAI-Y-158; GenBank JQ581045).

Comments – This species belongs to the *Filobasidiales* lineage. Based on its ability to degrade phenol, *C. phenolicus* may be used to assimilate different aromatic compounds (Fonseca et al. 2000).

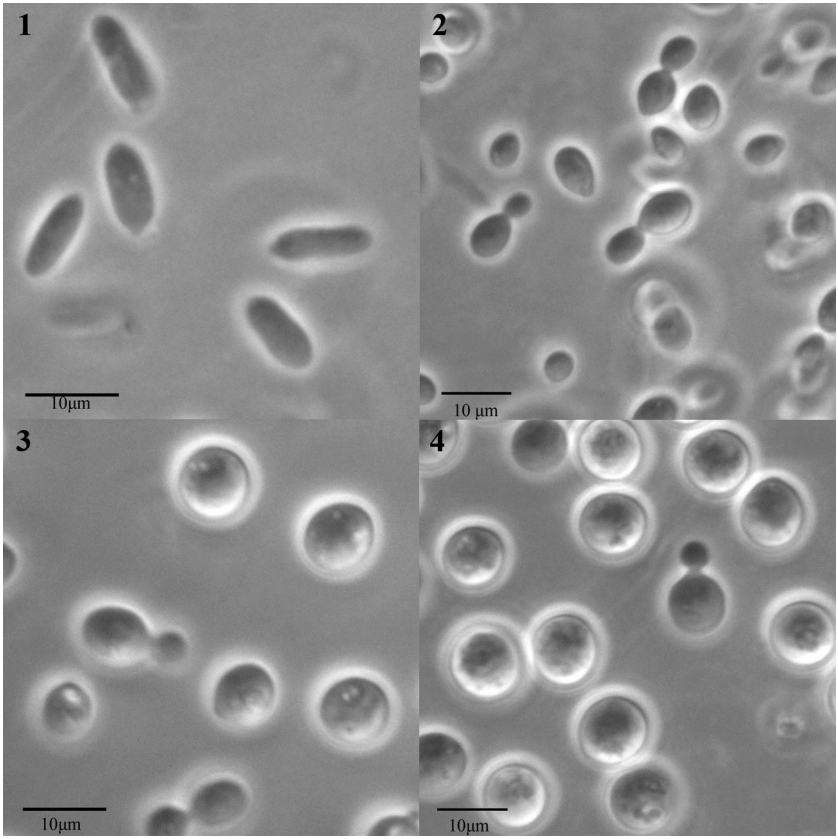
Distribution – Europe, Asia, South America, Africa (Hong et al. 2002, Cornelissen et al. 2003, Zachow et al. 2009, Vreulink et al. 2010, Mestre et al. 2011).

Cryptococcus terreus Di Menna, J. Gen. Microbiol. 11: 195 (1954) Fig. 4

Cells after 3 days growth on YM agar at 25°C single, mainly globose, 5–11 µm in diam; budding monopolar. Colonies [C/E]-colored, with glossy smooth surfaces.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′16″N 35″79′10″E, 11.I.2011, from grassland soil, leg. D.M. Gotman (HAI-Y-162; GenBank JQ581046).

Comments – This species is also in the *Filobasidiales* lineage with *C. phenolicus* and was isolated from the same habitat. Apparently, *C. terreus* is a soil inhabitant. As with *C. phenolicus*, *C. terreus* can assimilate phenol and phenol-related compounds (Bergauer et al. 2005). *Cryptococcus terreus*, *C. fuscescens,* and *C. phenolicus* are the main cryptococcal soil inhabitants (Kurtzman et al. 2011).



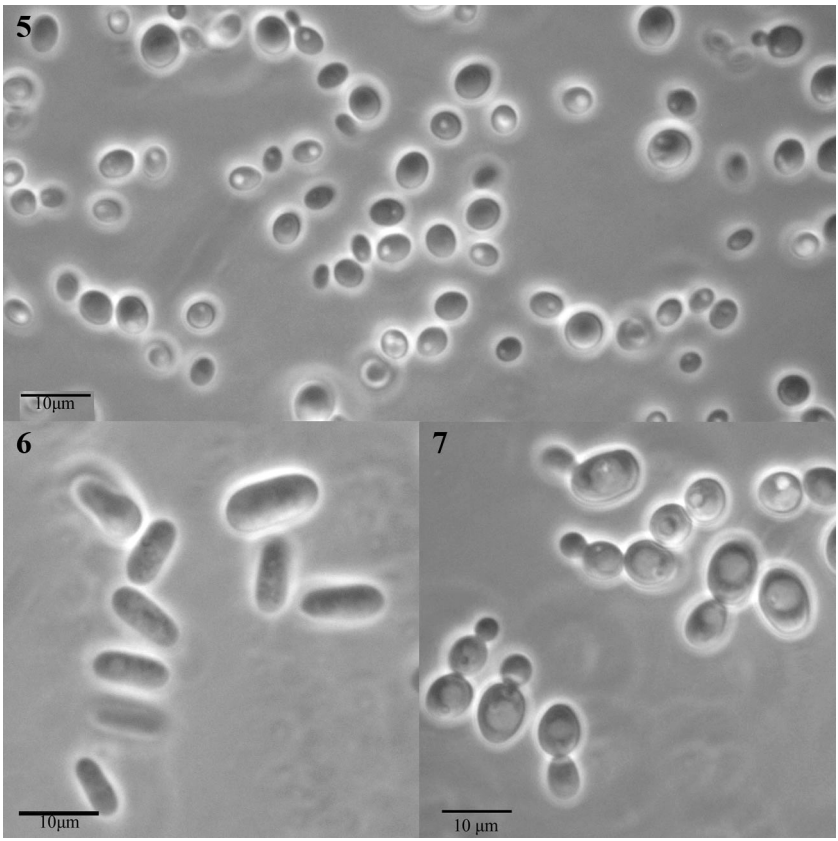
Figs 1–4. Yeast cells after two days of growing on YM medium, dark field light microscopy. 1. *A. nothofagi*; 2. *C. carnescens*; 3. *C. phenolicus*; 4. *C. terreus*. Scale bar = 10 µm.

Distribution – Europe, Asia, North America, South America, Oceania (Ba et al. 2000; Sláviková &Vadkertiová 2000; Hong et al. 2002, 2006; Maksimova & Chernov 2004; Mankowski & Morrell 2004; Bergauer et al. 2005; Langdon et al. 2005; Borelli et al. 2006; Lynch & Thorn 2006; Vishniac 2006; Rodrigues et al. 2009; Singh et al. 2009; Wang et al. 2009; Golubev & Tomashevskaya 2010; Mestre et al. 2011).

Komagataella pastoris (Guillierm.) Y. Yamada et al., Biosc., Biotechn., Biochem. 59(3): 444 (1995) Fig. 5

Cells after 3 days growth on YM agar at 25°C spherical to ovoid, 2–6 µm, occurring singly or in pairs; budding polar. Colonies white, mucoid.

Specimen examined – **ISRAEL. Carmel Mount**, Haifa, Carmel National Park, 32°75′03″N 35°02′77″E, 18.XII.2010, from soil under *Quercus calliprinos*, leg. D.M. Gotman (HAI-Y-67; GenBank JQ581044).



Figs 5–7. Yeast cells after two days of growing on YM medium, dark field light microscopy. 5. *K. pastoris*; 6. *R. lusitaniae*; 7. *S. occidentalis*. Scale bar = 10 µm.

Comments – *K. pastoris* represents *Phaffomycetaceae* in the *Saccharomycetales*. The major habitats of *K. pastoris* are tree exudates and decomposing wood. Phylogenetically, it is very close to *K. phaffii* and *K. pseudopastoris*, having no differences in fermentation and growth, but only in D1/D2 LSU rRNA gene sequences (Kurtzman 2005). They also were isolated from tree exudates and rotted wood. *K. pastoris* has a strong biotechnological potential as a recombinant protein production system (Heyland et al. 2010).

Distribution – Europe, North America, South America, Africa (Phaff & Knapp 1956; Faparusi 1981; Lachance et al. 1982, 1995; Spencer et al. 1995; Dlauchy et al. 2003).

Rhodosporidium lusitaniae Á. Fonseca & J.P. Samp., Syst. Appl. Microbiol. 15(1): 48 (1992) Fig. 6

Cells after 3 days growth on YM agar at 25°C single, cylindrical to bacilliform, 3–4 × 9–11 µm; budding polar. Colonies apricot colored, smooth.

Specimen examined – **ISRAEL. Carmel Mount**, Haifa, Carmel National Park, 32°75′00″N 35°02′70″E, 18.XII.2010, from fallen leaves of *Quercus calliprinos*, leg. D.M. Gotman (HAI-Y-58; GenBank JQ581047).

Comments – *Rhodosporidium lusitaniae* is classified in *Sporidiobolales* (*Microbotryomycetes*).

Distribution – Europe (Bergauer et al. 2005), where it is restricted to Austria, Israel, and Portugal.

Schwanniomyces occidentalis Klöcker, Meddn Carlsberg Lab. 7: 276 (1909) Fig. 7

Cells after several days growth on YM agar at 25°C globose to ovoid, 5–11 µm in diam., singly or in pairs and small chains; budding polar. Colonies white, smooth.

Specimen examined – **ISRAEL. Lower Galilee**, Kefar Kish, 32°65′45″N 35°44′44″E, 22.XII.2010, from soil, leg. D.M. Gotman (HAI-Y-90; GenBank JQ581050).

Comments – Almost all known strains of this species in the *Debaryomycetaceae* (*Saccharomycetales*) have been isolated from soil. *Schwanniomyces occidentalis,* which is regarded as an important "non-conventional" yeast with potential biotechnological value, has a good capacity for starch degradation. Several genetic models were developed for the production of heterologous proteins from *S. occidentalis* (Spencer et al. 2002).

Distribution – Europe, Asia, North America (Capriotti 1957, Phaff et al. 1960, Urano et al. 2002, Sláviková &Vadkertiová 2003, Boby et al. 2008).

Discussion

Our research has revealed seven yeast species new for Israeli mycobiota. Four were found in soil (*C. phenolicus*, *C. terreus*, *K. pastoris*, *S. occidentalis*), one on fallen leaves of *Quercus boissieri* (*C. carnescens*), and one on fallen leaves of *Quercus calliprinos* (*R. lusitaniae*). *Apiotrichum nothofagi* was isolated both from fallen leaves of *Ceratonia siliqua* and from fallen needles of *Pinus halepensis*. Five species are new for the Middle East. Only *C. terreus* and *S. occidentalis* were previously recorded in Egypt (Youssef & Eldin 1998, El-Assal et al. 2011).

Worldwide, *Cryptococcus terreus* and *Schwanniomyces occidentalis* have been isolated from various soil types and have wide geographical distribution. The cosmopolitan *C. carnescens* has been found on different substrates all over the world. Besides soil, *C. carnescens* has been isolated from grape surface, seawater, marine microalgae, leaves, needle litter, and even from such extreme habitats as

glacial ice. Soil and leaf litter are the main habitats for *Rhodosporidium lusitaniae*, which may possibly inhabit the entire Mediterranean region in addition to Israel and Portugal; its connection with Mediterranean forest ecosystems can be traced. *Komagataella pastoris*, associated with tree exudates in deciduous forests, has been recorded in different parts of the world. In Israel it was found in soil (under oak), an unusual habitat for the species; yeast cells may possibly have entered the soil from decayed oak wood. The habitat of *A. nothofagi* is not so clear, as the species has been recorded only a few times, mainly associated with forest litter and decayed wood. This species has also been isolated from seawater and blueberry fruit.

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